

Claims 20-23 (cancelled).

Listing of Claims:

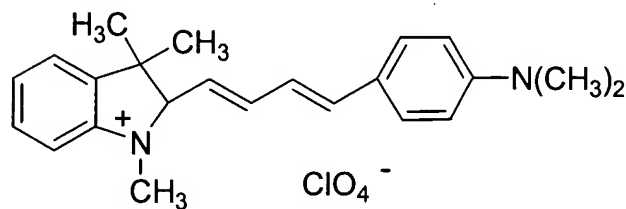
1. (Currently Amended) A method of staining bacteria in a sample comprising: adding ~~working~~ a polymethine dye to the ~~on a~~ sample in the presence of a substance capable of reducing nitrite ions ~~to stain bacteria in the sample~~.

2. (Original) A method according to claim 1, wherein the substance capable of reducing nitrite ions is selected from the group consisting of: ascorbic acid, isoascorbic acid, aminomethanesulfonic acid, aminoethanesulfonic acid, glutamic acid, aspartic acid, mercaptoacetic acid, 3-mercaptopropionic acid, sulfamic acid, sulfanilic acid, sulfurous acid, pyrosulfurous acid, phosphinic acid, glycine, glutamine, asparagine, methionine, glutathione, cysteine, hydroxylamine and salts thereof; sulfanilamide; aminomethane; mercaptoethanol; thiophenol and urea.

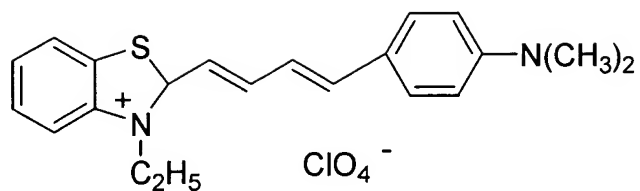
3. (Currently Amended) A method according to claim 1, wherein the polymethine dye is at least one selected from the following group consisting of:

(1) Thiazole Orange;

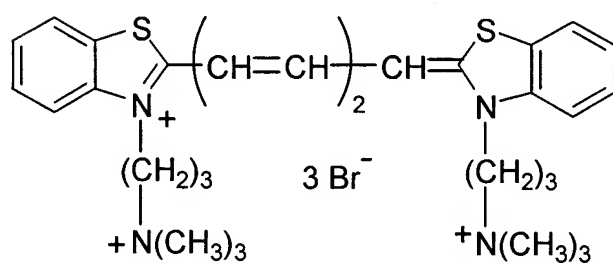
(2)



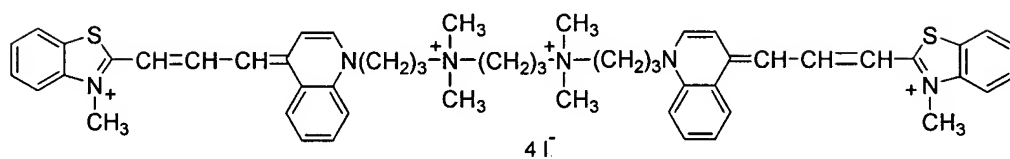
(3)



(4)

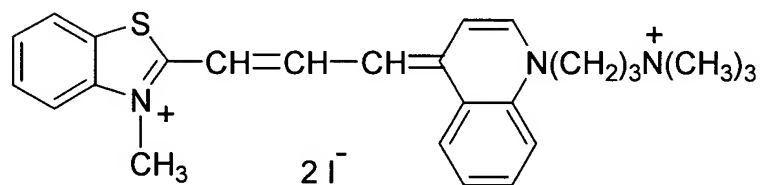


(5)



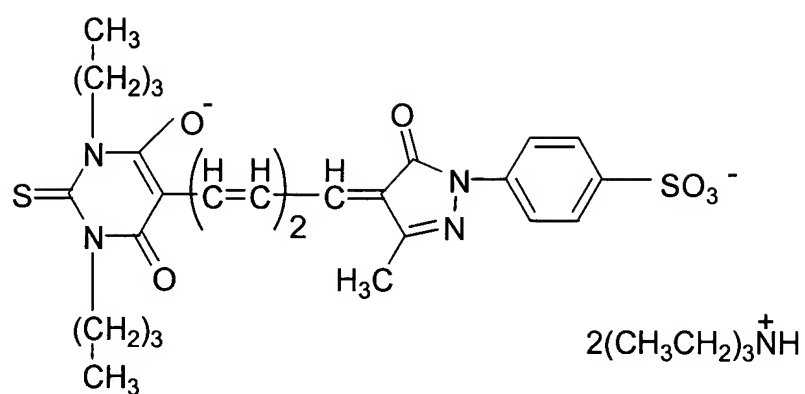
[(5)]

(6)

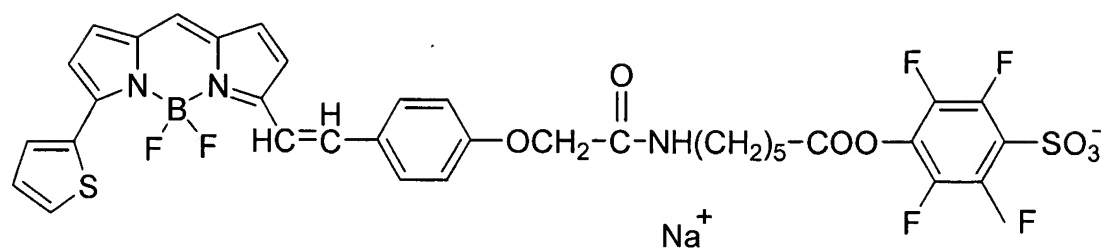


[(6)]

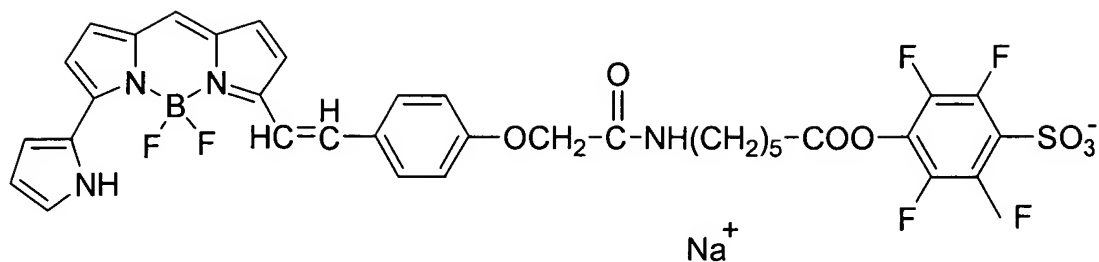
(7)



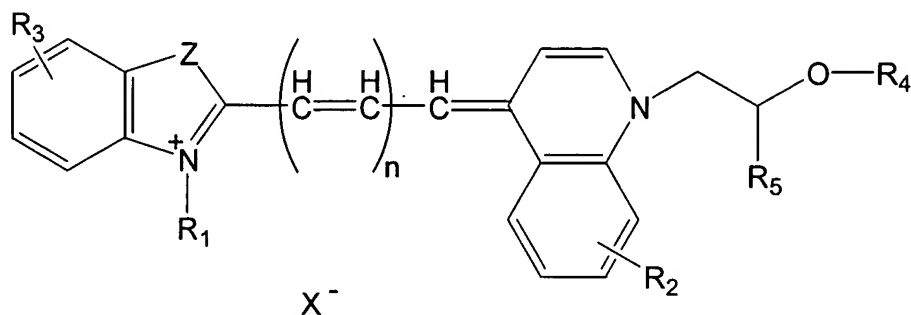
(8)



(9)

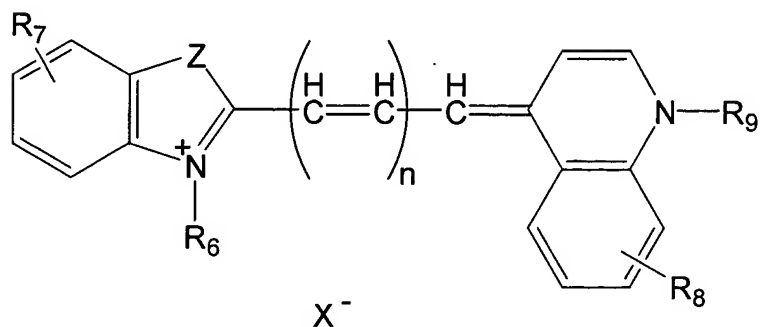


(10) a compound represented by the following general formula:



wherein R_1 is a hydrogen atom or a C_{1-3} alkyl group; R_2 and R_3 are a hydrogen atom, a C_{1-3} alkyl group or a C_{1-3} alkoxy group; R_4 is a hydrogen atom, an acyl group or a C_{1-3} alkyl group; R_5 is a hydrogen atom or a C_{1-3} alkyl group which may be substituted; Z is a sulfur atom, an oxygen atom or a carbon atom substituted with a C_{1-3} alkyl group; n is 1 or 2; X^- is an anion; and

(11) a compound represented by the following general formula:

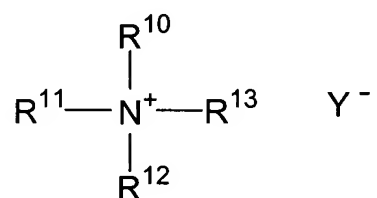


(11) a compound represented by the following general formula:

wherein R₁ is a hydrogen atom or a C₁₋₁₈ alkyl group; R₂ and R₃ are a hydrogen atom, a C₁₋₃ alkyl group or a C₁₋₃ alkoxy group; R₄ is a hydrogen atom, an acyl group or a C₁₋₁₈ alkyl group; Z is sulfur, oxygen or carbon having a C₁₋₃ alkyl group; n is 0, 1 or 2; ~~X⁺~~ X⁻ is an anion.

4. (Currently Amended) A method according to claim 1, ~~wherein the working is carried out in the existence with~~ presence of a cationic surfactant.

5. (Currently Amended) A method according to claim 4, wherein the cationic surfactant is a quaternary ammonium salt represented by the following formula:



wherein R¹⁰ is a C₆₋₁₈ alkyl group or (C₆H₅)-CH₂-; R¹¹, R¹² and R¹³, the same or different, are a C₁₋₃ alkyl group or a benzyl group; ~~Y⁺~~ Y⁻ is a halogen ion.

6. (Original) A method according to claim 5, wherein the quaternary ammonium salt is at least one selected from the group consisting of: decyl trimethyl ammonium salt, dodecyl trimethyl ammonium salt, tetradecyl trimethyl ammonium salt, hexadecyl trimethyl ammonium salt and octadecyl trimethyl ammonium salt.

7. (Currently Amended) A method according to claim 1, wherein the ~~dye is worked under~~ sample is in an acidic state.

8. (Original) A method according to claim 7, wherein the acidic state is set at pH 2.0-4.5.

9. (Currently Amended) A method according to claim 4 7, wherein a buffer of pKa 1-5.5 is used to maintain an acidic pH.

10. (Original) A method according to claim 9, wherein the buffer is at least one selected from the group consisting of: citric acid-NaOH, potassium dihydrogen phosphate-disodium hydrogen phosphate, potassium dihydrogen phosphate-NaOH, citric acid- disodium hydrogen phosphate, potassium hydrogen phthalate-NaOH, succinic acid-NaOH, lactic acid-NaOH, ε-aminocaproic acid-HCl, fumaric acid-HCl, β-alanine-NaOH and glycine-NaOH.

11. (Currently Amended) A method according to claim 1, ~~wherein the working is carried out in the existence with~~ presence of an inorganic salt of either sulfate or nitrate.

12. (Currently Amended) A method according to claim 1, wherein the dye is present in an amount of ~~worked at~~ 0.1 to 100 ppm in the sample.

13. (Currently Amended) A method according to claim 1, wherein the substance capable of reducing nitrite ions exists in the sample in such an amount that it can reduce[s] the nitrite ions produced by bacteria by ~~of~~ 10^5 to 10^8 /ml.

14. (Currently Amended) A method according to claim 4 ~~4~~, wherein the cationic surfactant exists at 10 to 30000 mg/l in the sample.

15. (Currently Amended) A method according to claim 10, wherein the ~~acid or the compound~~ buffer maintaining an acidic pH exists at 10 to 500 mM in the sample.

16. (Original) A method according to claim 1, wherein the sample is a urine, blood or spinal fluid.

17. (Currently Amended) The method according to claim 1, further comprising the steps of A ~~method of detecting and counting bacteria comprising the following steps of:~~

~~————— (1) working a polymethine dye on a sample by a method as described in any one of claims 1 to stain bacteria in the sample,~~

~~(2) (1) introducing the thus-treated sample containing the stained bacteria into a detecting part of a flow cytometer and irradiating cells of the stained bacteria one-by-one with light to measure scattered light and fluorescent light emitted from each of the cells the stained bacteria; and~~

~~(3) (2) discriminating the bacteria from other components in accordance with an intensity of a scattered light signal and an intensity of a fluorescent light signal or a pulse width reflecting the length of particles to counting the number of the stained bacteria.~~

18. (Currently Amended) A method according to claim 17, wherein the step of staining the bacteria ~~(1)~~ is carried out in the presence of ~~by the steps of~~

~~—— (a) mixing a sample with an aqueous solution containing a substance capable of reducing nitrite ions and/or a cationic surfactant [to accelerate dye transmissivity of bacteria;~~

~~—— (b) staining the bacteria for a predetermined period with a polymethine dye;~~

19. (Currently Amended) A method according to claim 17, wherein the step of ~~(3)~~ ~~of discriminating and counting the stained bacteria is carried out by determining the parameters selected from the group consisting of in accordance with at least one selected from the following combinations of:~~

(i) a forward scattered light intensity and a forward scattered light pulse width;

(ii) a forward scattered light intensity and a fluorescent light intensity; and

(iii) a forward scattered light pulse width and a fluorescent light intensity.

20. (Canceled)

21. (Canceled)

22. (Cancelled)

23. (Canceled)